THEORETICAL BASIS OF MULTIPLE CHEMOTHERAPY FROM BACTERIOLOGICAL ASPECTS

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It may be the greatest problem in chemotherapy how the development of drug-resistant strains of bacteria may be prevented or delayed. Considering on the origin of drug-resistant bacteria[1~5], it appears almost impossible to prevent completely the mutation of drug-resistant bacteria. Accordingly, it is the problem remained for us how to delay the development. It is now well known that successive use of single drugs causes rapidly the development of drug-resistant bacteria and combined use of two or three drugs delay it. The knowledge has been given by many fundamental and clinical studies especially on the chemotherapy of tuberculosis. It is the purpose of the present paper to add some considerations, based on the studies on the chemotherapy of tuberculosis made, on the mechanism of delaying the development of drug-resistant bacteria by multiple chemotherapy and to suggest the possible best method of administration of drugs.

Mechanism of delaying the development of drug-resistant bacteria by multiple chemotherapy

The mechanism is indicated from various points of view as follows :

1) Mutation rate (DEMEREC[1]; KLEIN, et al.[6~8]; SZYBALSKI[9]).

The first factor consists of the selective elimination of bacteria resistant to one drug by means of another drug. The spontaneous occurrence of mutants resistant to two drugs unrelated is very rare. Theoretically, the probability of double mutation is the product of individual rates.

2) Growth rate

The development of drug-resistant bacteria means the development of a drug-resistant bacterial population (a drug-resistant strain), sometimes all of which and sometimes most of which consists of drug-resistant bacteria. The development of a drug-resistant population depends not only on the mutation rate but also on the growth rate of drug-resistant bacteria.

Generally speaking, there appears no drug as completely inhibitory for the growth of sensitive bacteria and there appears also no resistant bacteria as its growth not influenced by the drug to which the bacteria are resistant. Most drugs retard significantly the growth of sensitive bacteria and less

significantly the growth of resistant bacteria and the effect of drugs is, therefore, rather quantitative than qualitative between sensitive and resistant bacteria. It is found that the following drugs have more or less a retarding effect on the growth of bacteria sensitive as well as resistant : streptomycin[10,11], PAS[12,13], 4-acetylaminobenzal thiosemicarbazone[14], viomycin[15], sulfa drugs[16~18]. It may be said that only a difference between growth rates of drug-resistant and drug-sensitive bacteria acts as a selective agent in the presence of a given drug. Consequently, the velocity of the development of drug-resistant population might vary depending on the difference.

If assumed that the mutation rate to drug-resistance is m/N per bacterium per generation time, a bacterial population consisting of bacterial number N would contain approximately m of resistant mutants. If the population is exposed to a given bacteriostatic drug designated as drug A, in which sensitive bacteria have the generation time G_0 and resistant mutants have the generation time G (G_0 is larger than G), ratio K of the number of resistant mutants per the number of sensitive bacteria after an incubation period t would be given by the following equation :

$$K = am \, 2t/G / N \, 2t/G_0$$

Here, $am \, 2t/G$ and $N \, 2t/G_0$ are the number of resistant mutants and the number of sensitive bacteria after an incubation period t. The number a has been derived as follows : It is expected that m of resistant mutants that had been contained in the population before incubation might multiply and reach the number $m \, 2t/G$ after t hours. On the other side, new resistant mutants might be mutated from sensitive population that would multiply and reach the number $N \, 2t/G_0$ after t hours. Multiplication of the number " a " has been derived from calculating the resistant mutants newly mutated from sensitive population. It varies from 1 to t/G depending on the ratio of the generation times G_0 and G, and, if the generation time is constant, the value " a " is also constant. It is the highest ($a = t/G$), if the generation time G_0 and G are similar to each other, it is less than t/G, if the generation time G_0 is larger than the generation time G, and it is approximately 1, if the generation time G_0 is very larger than the generation time G. In most

cases of the presence of drugs, it may be regarded as of the last case ($a=1$). The equation is changed as follows .

$$t = ((\log K - \log am/N)/\log 2) \times (G_0 G/(G_0 - G))$$

Consequently, the time t required for obtaining a constant ratio K varies depending on the generation times G_0 and G. Therefore, the following considerations may be given.

(a) The greater the generation time G_0 and G, the greater the time (The less the growth rates of sensitive as well as resistant bacteria, the more retarded the development of drug-resistant population).

(b) The greater the difference between the generation time G_0 and G, the less the time.

(c) If there co-exists another drug B that retards both growth rates of sensitive and resistant bacteria at the same rate, the time becomes greater, i. e., the development of population resistant to drug A is retarded.

It is known that sulfa drugs represent only a slight or no clinical effect on tuberculosis when used alone. However, it has been indicated by us[19] that the drugs are effective for retarding the development of isoniazid-resistance in tuberculous patients. The effect is considered to be derived from their growth-retarding effect on tubercle bacilli, for the drugs used appeared never selective for sulfa drus-resistant mutants in human bodies.

It is also well known that PAS is most effective for retarding the development of streptomycin-resistant population[20~23] and also effective for that of isoniazid-resistant population[24~28] in tubercle bacilli. However, theoretically considering, it is possible that PAS might be still effective even in such a small amount as moderately retarding the growth rate of the organism.

(d) The development of drug-resistant population is more retarded in tuberculous productive lesions, where the growth rate of bacteria is lower, and it is faster, in tuberculous cavities, where the growth rate is higher. This consideration agrees well with the facts already observed by many investigators[29~31].

(3) Population structure

The population structure of tubercle bacilli occurring in patients is in most cases heterogeneous with respect to isoniazid-resistance as well as PAS-resistance, while it is frequently homogeneous with respect to streptomycin-resistance[32,33]. The population structure of M. tuberculosis var. hominis is still heterogeneous with respect to triple drug-resistance (streptomycin-, isoniazid-, and PAS-resistance) even after in vitro multiple-step selection with the drugs simultaneously used[34].

The existence of the heterogeneity of population

structure suggests that the administration of multiple drugs might be still effective even after establishment of multiple-resistant strains.

In addition to the above, the problem may be discussed with respect to viability duration of bacteria which had grown on subinhibitory concentrations of single or multiple drugs and with respect to collateral sensitivity. It appears, in the present state of studies, not yet evident how these take a part in multiple chemotherapy.

Some theoretical considerations on the *in vivo* development of multiple-drug-resistant bacteria and on the method of administration of drugs

I wish to discuss this problem taking the mechanism of in vivo establishment of multiple-drug-resistant population (strain) in tubercle bacilli as an example.

Mutants resistant to 10 mcg of streptomycin is found among a parent strain of M. tuberculosis var. hominis not proviously exposed to the drug at a rate of approximately 10^{-7}, mutants resistant to 1 mcg of PAS is done at a rate of approximately 10^{-5}, and mutants resistant to 0.1 mcg of isoniazid at a rate of approximately 10^{-6} Consequently, it is considered that mutants simultaneously resistant to both streptomycin and PAS appear at a rate of 10^{-12}, mutants resistant to both streptomycin and isoniazid at a rate of 10^{-13}, mutants resistant to both isoniazid and PAS at a rate of 10^{-11}, and mutant resistant to the three drugs at a rate of 10^{-18}. If one mg of the organism consisted of 10^7 of viable cells, 10^{12} of viable cells correspond to 100 g and 10^{18} do to 10^5 kg. Therefore, it is sure, as mentioned by SZYBALSKI[9], that there is not any triple-resistant mutants within human body before administration of drugs, and it is very probable that there is not any double-resistant mutants within it. Nevertheless, the development of triple-resistant population can occur even with triple-combined chemotherapy with streptomycin, isoniazid and PAS, although the rate of the development is much lower by triple-combined chemotherapy than by successive use of single drugs[35~39]. It is desirable, therefore, to know the reason why the development does occur.

Let us consider the relationship between the mutation rate and the size of total bacterial population in human body. We designate mutation rates to drugs A, B, C, ···, respectively, as 10^{-a}, 10^{-b}, 10^{-c}···, respectively. The mutation rate to multiple-drug-resistance must be $10^{-(a+b+c+\cdots)} = 1/10^{(a+b+c+\cdots)} = M^{-1}$. Here, $M = 10^{(a+b+c+\cdots)}$ and M varies depending on drug concentrations. The mutation rate to triple-drug-resistance is $10^{-(a+b+c)}$ and, therefore, M is $10^{(a+b+c)}$ in this case. Moreover, we designate the number of total bacterial population as N.

If N is greater than M, the development of multiple-drug-resistant population occurs unrelatedly with the method of administration of drugs. For example, if N is 10^{20}, N contains 10^2 ($10^{20} - 10^{18} = 10^2$) of triple-resistant mutants. If assumed that, to simplify the consideration, resistant bacteria had the generation time G in the presence of drugs and the generation time G equals to the generation time of sensitive bacteria in the absence of drug (resistant bacteria had the same generation time in the presence of drugs as did sensitive bacteria in the absence of drug), the time required for these one hundred triple-resistant mutants to gain the original size of population (10^{20}) in the presence of triple drugs is calculated as follows : $10^2 \times 2\,t/G = 10^{20}$, that is, $t = 18\,G/\log 2$.

Therefore, if triple-chemotherapy is made against a bacterial population consisting of 10^{20} of viable cells, the population is changed into a population consisting of 10^{20} of triple-resistant bacteria after a period of $18\,G/\log 2$. On the other side, if the same population is treated by successive administration of single drugs, the time required for obtaining the size of 10^{20} of triple-resistant bacteria is considered as follows : If streptomycin at first administrated, 10^{13} ($= 10^{20} - 10^7$) of streptomycin-resistant mutants contained in the population can multiply and reach the original size after the time $t = 7\,G/\log 2$. If isoniazid is as second admistrated against the streptomycin resistant population, 10^{14} ($= 10^{20} - 10^6$) of streptomycin-and isoniazid-resistant mutants contained in the population can multiply and reach the original size after the time $t = 6\,G/\log 2$. If PAS is at third administrated against the streptomycin-and isoniazid-resistant population, 10^{15} ($= 10^{20} - 10^5$) of triple-resistant mutants contained in the double-resistant population can multiply and reach the original size after the time $t = 5\,G/\log 2$. Therefore, the time required for the original population to change into the triple-resistant population by successive use of the three drugs is calculated as $7\,G/\log 2 + 6\,G/\log 2 + 5\,G/\log 2 = 18\,G/\log 2$, and it is the same as obtained previously by simultaneous use of three drugs.

As shown above, it is considered that, if N is greater than M, the time required for the development of triple (or multiple)-resistant population is always the same one indifferent from the method of administration of drugs.

On the other side, if N is smaller than M, the development of triple-resistant population theoretically should not occur. Indeed, the size of N in human body appears not so much great as surpassing M. Nevertheless, it is sometimes seen the development of triple-resistant population by triple chemotherapy of tuberculosis. It indicates that M

is not always constant and sometimes decreases. It means that drug concentrations in human body frequently decrease or diminish and sometimes only one drug remains in foci. If one drug A remained in foci, the development of drug-resistance to drug A readily occurs, for, in this case, M is 10^0 and N is probably greater than M. If drug-resistance is thus established to the drug A, the development of drug-resistance to another drugs B and C would occur as easily as only the drugs B and C administrated, ever when three drugs were simultaneously used. (*If more accurately mentioned, this is not true, for mutants resistant to drug A is never uninfluenced by the drug A. See supplement 2). Thus, multiple drug-resistance appears to occur successively.

The considerations above described appear to give us several important clinical suggestions.

(a) If N is greater than M, the development of multiple drug-resistance readily occurs indifferently of the method of administration of drugs. If N is smaller than M, the development of multiple drug-resistance is delayed or prevented by the method of administration giving such M.

In orded to delay the development of drug-resistant population, it is necessary to maintain drug concentrations enough higher than M. Therefore, it is desirable that administration of multiple drugs is made to obtain the simultaneous peak of drug concentrations in foci.

(b) If N is relatively great, it is necessary to use enough great M, i. e., to use multiple chemotherapy. However, if N is relatively small, it needs not always to use multiple chemotherapy and a sufficient effective chemotherapy can be performed even by one drug or a combination of two drugs.

(Supplement 1) The chemotherapy of cancer also would be discussed from the same points of view. The resection of cancer-tumor means to making smaller the size of N. Nevertheless, even unoperable small tumors would contain N enough large to contain drug-resistant mutants. Therefore, the chemotherapy of cancer would be established only by multiple chemotherapy as has been established the chemotherapy of tuberculous cavities by multiple chemotherapy.

(Supplement 2) Even if any drug-resistance had occurred during the course of chemotherapy, the administration of " resistance-drugs " (drugs to which bacteria are resistant) should be continued, for (1) " resistance-drugs " delay more or less the growth rate of resistant bacteria[16~18] and, therefore, may be more or less effective for delaying the development of drug-resistance to another drugs ; (2) the composition of drug-resistant population may be heterogeneous with respect to drug-resist-

ance, especially to multiple drug-resistance[34], and, therefore, the administration of multiple "resistance-drugs" may be more or less effective for reducing the size of N; (3) it is known that PAS-resistant tubercle bacilli[40] and isoniazid-resistant tubercle bacilli[41~43] are of less virulence and, therefore, the administration of "resistance-drugs" may be effective for reducing the virulence.

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